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Synthesis and Evaluation of Novel Chemical Compounds for Weed Management

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To the Graduate Council:

I am submitting herewith a thesis written by Joseph Warden Thomas entitled "Synthesis and Evaluation of Novel Chemical Compounds for Weed Management." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

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Synthesis and Evaluation of Novel Chemical Compounds for Weed Management

A Thesis Presented for

the Master of Science

Degree

The University of Tennessee, Knoxville

Joseph Warden Thomas

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DEDICATION

I would like to dedicate this document to my parents, Peter and Natalie, without whom I would not have been able to produce it.

ACKNOWLEDGEMENTS

A project of this size and complexity could not have been done without the expertise and hard work of many individuals. I would like to thank the members of my committee; Dr. Gregory R. Armel, Dr. James T. Brosnan, Dr. Michael D. Best, Dr. William E. Klingeman, and Dr. Dean A. Kopsell. A special thanks is due to Dr. Armel for his creative mind and passion for the subject of herbicide science. I would also like to thank Dr. Brosnan for his writing and organizational experience. Both deserve credit for their dedication and patience towards me and the project.

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A wise man once told me “We stand on the shoulders of giants.” I am grateful to all the giants I have met at the University of Tennessee.

ABSTRACT

Options for controlling herbicide resistant weed species are severely limited; thus, the recent proliferation of these species is a significant concern to land managers. The discovery and development of novel herbicidally active compounds is one method proposed to manage herbicide resistant weed species. Novel herbicides would provide effective new options for control of existing weed species, and alleviate the narrow selection pressure that leads to the development of herbicide resistance.

Research examined analogs of the synthetic cytokinin thidiazuron (TDZ). TDZ is used as a pre-harvest defoliation of cotton (*Gossypium hirsutum* L.) and as a plant growth regulator. Although the use of TDZ as a plant growth regulator in tissue culture systems has been extensively studied there is no available data on weed susceptibility to TDZ. Twenty seven analogs of TDZ were synthesized at the University of Tennessee (Knoxville, Tennessee). Thidiazuron and these analogs were applied postemergence corn (*Zea mays* L.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), field bindweed (*Convolvulus arvensis* L.), barnyardgrass (*Echinochloa crus-gali* (L.) P.Beauv.), velvetleaf (*Abutilon theophrasti* Medik.), and redroot pigweed (*Amaranthus retroflexus* L.). at 250 g ha^{-1} [grams per hectare]. TDZ injured velvetleaf and redroot pigweed 80 to 96% while only inducing 0 to 2% injury to corn. Across all species tested, minimal injury was induced by any of the analogs synthesized. Results indicate that the synthetic cytokinin, TDZ, may have utility for weed management in corn.

Additional research examined preemergence control of large crabgrass (*Digitaria sanguinalis* (L.) Scop.), giant foxtail (*Setaria faberi* Herrm.), and common purslane (*Portulaca oleracea* L.) with analogs of the cellulose biosynthesis inhibitor dichlobenil. Treatments consisted of two pyridine and one pyrimidine heterocyclic analogs applied in comparison to

dichlobenil. Japanese holly (*Ilex crenata* Thunb.) tolerance was monitored following postemergence over-the-top applications in addition to preemergence weed control efficacy. All treatments were applied at 1,5, and 10 kg ha^{-1}. Only the pyrimidine analog controlled common purslane and large crabgrass similar to dichlobenil at all rates evaluated. Additional research should be performed to determine if this pyrimidine analog inhibits cellulose biosynthesis at sites of action similar to dichlobenil.

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INTRODUCTION

This thesis is divided into two chapters: 1) a chapter presenting the results of research evaluating postemergence weed control efficacy and crop tolerance following applications of potential synthetic cytokinins; and 2) a chapter presenting the results of research evaluating preemergence weed control efficacy and crop tolerance following applications of pyridine and pyrimidine analogs of the commercial herbicide dichlobenil. Both chapters have been prepared for submission to the *Journal of Pesticide Science*. My contributions to each chapter include (i) conducting the experiments, (ii) collecting, processing and collaborating on data analysis and interpretation, (iii) reading literature, (iv) and preparing the manuscript.

CHAPTER I

HERBICIDAL ACTIVITY OF POTENTIAL SYNTHETIC CYTOKININS

This chapter is based on a paper to be submitted for publication by Joseph W. Thomas, Gregory R. Armel, Michael D. Best, James T. Brosnan, Dean A. Kopsell, Jose J. Vargas, and Chi-Linh Do-Tahnh.

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My contributions to this paper include (i) conducting the experiments, (ii) collecting, processing and collaborating on data analysis and interpretation, (iii) reading literature, (iv) and collaborating on the manuscript.

ABSTRACT

Thidiazuron (**TDZ**) is a synthetic cytokinin used as a plant growth regulator and pre-harvest cotton (*Gossypium hirsutum* L.) defoliant. Data describing weed control efficacy of **TDZ** applications are limited. Compounds with structural similarity to **TDZ**, such as N-(cyclohexylmethyl)-4-methyl-thiadiazole-5-carboxamide (**TN1**) may also exhibit herbicidal activity; however, minimal data are available regarding the efficacy of synthetic cytokinins for weed management. Two series of **TN1** analogs were synthesized and evaluated for herbicidal activity at the University of Tennessee (Knoxville, TN) in 2012. A non-treated check, **TN1**, and **TDZ** were included for comparison. All compounds were applied postemergence at 250 g ha^{-1} to corn (*Zea mays* L.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), field bindweed (*Convolvulus arvensis* L.), barnyardgrass (*Echinochloa crus-gali* (L.) P.Beauv.), velvetleaf (*Abutilon theophrasti* Medik.), and redroot pigweed (*Amaranthus retroflexus* L.). **TDZ** injured velvetleaf and redroot pigweed 80 to 96% while only inducing 0 to 2% injury to corn. Across all species tested, minimal injury was induced by any of the analogs synthesized. Results indicate that the synthetic cytokinin, **TDZ**, may have utility for weed management in corn. Future research should evaluate crop tolerance and weed control with **TDZ** as it could provide growers a new mode of action.

INTRODUCTION

Herbicide resistant weed species are currently a very important issue in weed science. There are now 217 species of herbicide resistant weeds, many of which have developed cross-resistance to multiple herbicide chemistries (Heap 2013; Vencill et al. 2012). For example, *Amaranthus tuberculatus* (Moq.) J.D.Sauer populations surveyed in Illinois and Missouri were resistant to both enolpyruvyl shikimate-3-phosphate synthase inhibitors and acetolactate synthase inhibitors, with some populations resistant to inhibitors of photosystem II and protoporphyrinogen oxidase as well (Tranel et al. 2011). Growers need to use products judiciously to protect the long-term effectiveness of the various modes and sites of action targeted by herbicides for weed management (Beckie 2006; Mortensen 2012). Discovering new herbicidal active ingredients, particularly those utilizing alternative modes of action, would aid in preserving the long-term effectiveness of herbicide options available to growers by reducing selection pressure for herbicide resistant weeds (Vencill et al. 2012). Additionally, new herbicidal active ingredients utilizing alternative modes of action may provide growers options for controlling herbicide resistant weeds (Duke 2012; Tranel et al. 2010).

Cytokinins are a class of phytohormone that affect plant processes such as organ formation, seed germination, cell development, and senescence (Hwang 2012). Mok et al. (1982) and Takasashi et al. (1978) demonstrated that thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea; **TDZ**) is a synthetic cytokinin, as it induces callus tissue formation similar to cytokinins in lima bean (*Phaseolus lunatus* L.) and tobacco (*Nicotiana tabacum* L.). Capelle et al. (1983) reported that the size of callus produced following **TDZ** treatment to lima beans was 30 times larger than with zeatin. Morphological changes to several plant species have been documented following **TDZ** applications (Murthy et al. 1998, Guo et. al. 2011). Huetteman and Preece (1993)

documented that **TDZ** induced formation of multiple shoots and adventitious buds in 39 different woody plant species. Adventitious roots and shoot formation has also been observed on geraniums (*Pelargonium x hortorum* Bailey cv. Kim and cv. Shone Helena) and ginseng (*Panax quinquefolium L.*) after applications of **TDZ** (Sango et al. 1995, Proctor et al. 1996). When applied alone, or in combination with auxin, **TDZ** can signal the conversion of somatic tissue to embryogenic tissue in several species including peanut (*Arachis hypogaea* L.), tobacco, and grapes (Vitis vinifera L.) (Murthy et al. 1998, Acanda et al. 2013).

TDZ is used as a growth regulator in plant tissue culture applications as well as a pre-harvest cotton (*Gossypium hirsutum* L.) defoliant sold under the trade name Dropp®SC (Guo 2011, Anonymous 2012). The defoliant properties of **TDZ** on cotton were first described by Arndt (1976). In Malvaceae species **TDZ** induces leaf abscission (Mok 1987). By mimicking the activity of cytokinins **TDZ** induces abscission of cotton leaves by increasing ethylene production (Suttle 1985, Grossmann 1991).

Weed control efficacy of **TDZ** is currently not well known. Moreover, other compounds that mimic cytokinin activity similar to **TDZ** may provide new options for weed management. Several patents claim that compounds used for defoliation of cotton are herbicidally active (Arndt 1979, Kruger 1982a, Kruger 1982b, and Rusch 1983). There are structural similarities between **TDZ** and the herbicidal compounds referenced in these patents. For example, one of the compounds demonstrating the greatest herbicidal activity in the patent literature, N-(cyclohexylmethyl)-4-methyl-thiadiazole-5-carboxamide (**TN1**), consists of an amide bridge linking two cyclic structures with only one small substituent group. **TDZ** is similar in that it contains a urea bridge between two cyclic structures but has no substituent groups (Figure 1.1). Analogs of **TN1** may exhibit herbicidal activity and thus allow agricultural producers to take

advantage of the underutilized synthetic cytokinin mode of action for weed management. The objective of this research was to synthesize analogs of **TN1** and test these compounds for herbicidal activity.

MATERIALS AND METHODS

Two series of analogous compounds were synthesized at the University of Tennessee (Knoxville, TN) and evaluated for herbicidal activity in greenhouse experiments. Analogs were divided into two series to independently examine effects of structural changes to distinctly different regions of each molecule.

Series 1 compounds contained variable linkage between the two cyclic structures of **TN1**. One common theme of the analogs from Series 1 was the incorporation of an oxygen atom into the linkage region as seen in the ester (**TN2**) and the hydroxylamine (**TN3**) analogs. Remaining analogs focused on the inclusion of branching alkyl substituent groups to the carbon atom in the linkage of **TN1**. These compounds included **TN4**, **TN5**, **TN6**, and **TN7**. Analogs **TN5** and **TN6** were stereoisomers that were included to determine if there was a difference in herbicidal activity between stereoisomers that could be exploited to maximize herbicidal activity. **TN4** was a racemic mix of the stereoisomers **TN5** and **TN6.** The **TN10** and **TN11** analogs differed from the base molecule **TN1** in that they were comprised of a benzyl ring structure rather than a cyclohexyl ring structure. **TN10** and **TN1** were included as a comparison to **TN4** and **TN7**, respectively, because preliminary experiments indicated that structures containing benzyl and cyclohexyl ring structures exhibited similar herbicidal activity.

Series 2 was composed of compounds with variable cyclic structures of the methyl substituted thiadiazole ring of **TN1**. Several analogs in Series 2 were very similar to **TN1**. **TN12**

and **TN13** had the sulfur atom of their thiadiazole ring located two carbons away from the carboxamide linkage as opposed to one carbon away in **TN1**. **TN12** also contained a substituent methyl group like **TN1** that **TN13** did not. A methyl substituted triazole was the ring structure found in the **TN14** analog and was included to examine the importance of the sulfur molecule in the ring structure (Figure 1.3). Compounds **TN21, TN22**, and **TN16** were included to determine the effect of oxygen atoms placed at different positions in the thiadiazole ring structure on herbicidal activity. Compounds with different substituent groups along the thiadiazole ring structure were also tested including an isopropyl group (**TN15)** and a bromine atom (**TN19)**. **TN23** and **TN24** were chosen to determine if six membered rings demonstrated differential herbicidal activity from five membered rings. Additionally, **TN23** contained one less carbon in the linkage region in order to keep overall molecule size similar to **TN1**. Similar to compounds from Series 1, benzyl versions of **TN1**, **TN23** and **TN24** were also evaluated in Series 2. These compounds were coded **TN25**, **TN26** and **TN27** respectively (Figure 1.4).

Compounds from both series were synthesized using the same general scheme (Figure 1.2). General synthesis of Series 1 and Series 2 compounds was accomplished by reacting a carboxylic acid with an alcohol or an amine, respectively, using carbodiimide mediated amide formation (Montalbetti and Falque 2005). Starting materials for the reactions were procured from commercial sources (Acros Organics, Geel, Belgium; Alfa Aesar, Ward Hill, MA; Matrix Scientific, Columbia, SC; Princeton BioMolecular Research, Inc., Princeton, NJ; Sigma Aldrich Co., St. Louis, MO; ThermoFisher Scientific, Waltham, MA).

For each reaction, one equivalent of a carboxylic acid was solubilized in dichloromethane. To this solution was added 1.1 equivalents of hydroxybenzotriazole, 1.3 equivalents of the alcohol or amine, and 1.1 equivalents of N,N-diisopropylethylamine

(Beyermann et al. 1991). The solution was then cooled to 0°C and 1 equivalent of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide was added. The mixture was returned to room temperature and stirred overnight. Then the solvent was removed under pressure and the resulting product was purified using silica gel flash chromatography. Solutions used for purification ranged from 20% to 50% ethyl acetate mixed with hexanes. Yields for these reactions ranged from 42% to 86% depending on the compound synthesized. Nuclear magnetic resonance (NMR) spectra of final products were confirmed using a Varian Gemini 300 MHz spectrometer (Agilent Technologies, Santa Clara, CA).

Synthesis of Series 1 Analogs

Specific synthesis of analogs from Series 1 was accomplished using the following compounds and the general procedure described above (Figure 1.3).

N-(cyclohexylmethyl)-4-methyl-thiadiazole-5-carboxamide (TN1). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and cyclohexylmethanamine (260 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

cyclohexylmethyl 4-methylthiadiazole-5-carboxylate (TN2). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and cyclohexylmethanol (260 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexoxy)-4-methyl-thiadiazole-5-carboxamide (TN3). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and O-cyclohexylhydroxylamine (260

mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(1-cyclohexylethyl)-4-methyl-thiadiazole-5-carboxamide (TN4). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and 1-cyclohexylethanamine (290 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-[(1R)-1-cyclohexylethyl]-4-methyl-thiadiazole-5-carboxamide (TN5). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and (1R)-1-cyclohexylethanamine (290 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-[(1S)-1-cyclohexylethyl]-4-methyl-thiadiazole-5-carboxamide (TN6). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and (1S)-1-cyclohexylethanamine (290 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(1-cyclohexyl-1-methyl-ethyl)-4-methyl-thiadiazole-5-carboxamide (TN7). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and 2-cyclohexylpropan-2-amine (320 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(1-ethynylcyclohexyl)-4-methyl-thiadiazole-5-carboxamide (TN8). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and 1-ethynylcyclohexanamine (280 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

4-methyl-N-phenethyl-thiadiazole-5-carboxamide (TN9). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and 2-phenylethanamine (280 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

4-methyl-N-(1-phenylethyl)thiadiazole-5-carboxamide (TN10). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and 1-phenylethanamine (280 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

4-methyl-N-(1-methyl-1-phenyl-ethyl)thiadiazole-5-carboxamide (TN11). To synthesize this compound 4-methylthiadiazole-5-carboxylic acid (300 mg, 2.08 mmol) and 2-phenylpropan-2-amine (310 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

Synthesis of Series 2 Analogs

Specific synthesis of analogs from Series 2 was accomplished using the following compounds and the general procedure described above (Figure 1.4).

**N-(cyclohexylmethyl)-5-methyl-1$l^4-thia-2,3-diazacyclopenta-1,4-diene-4-carboxamide (TN12).** To synthesize this compound 5-methyl-1$l^4-thia-2,3-diazacyclopenta-1,4-diene-4-carboxylic acid (300 mg, 2.05 mmol)
and cyclohexylmethanamine (260 mg, 2.26 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

**N-(cyclohexylmethyl)-1$l^4-thia-2,3-diazacyclopenta-1,4-diene-4-carboxamide (TN13).** To synthesize this compound 1$l^4-thia-2,3-diazacyclopenta-1,4-diene-4-carboxylic acid (300 mg,

2.27 mmol) and cyclohexylmethanamine (280 mg, 2.50 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)-4-methyl-1H-triazole-5-carboxamide (TN14). To synthesize this compound 4-methyl-1H-triazole-5-carboxylic acid (300 mg, 2.36 mmol) and cyclohexylmethanamine (290 mg, 2.60 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)-2-isopropyl-4-methyl-thiazole-5-carboxamide (TN15). To synthesize this compound 2-isopropyl-4-methyl-thiazole-5-carboxylic acid (300 mg, 1.62 mmol) and cyclohexylmethanamine (200 mg, 1.78 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)-4-methyl-oxazole-5-carboxamide (TN16). To synthesize this compound 4-methyloxazole-5-carboxylic acid (300 mg, 2.36 mmol) and cyclohexylmethanamine (290 mg, 2.60 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)thiophene-3-carboxamide (TN17). To synthesize this compound Thiophene-3-carboxylic acid (300 mg, 2.34 mmol) and cyclohexylmethanamine (290 mg, 2.58 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)-3-methyl-3H-thiophene-2-carboxamide (TN18). To synthesize this compound 3-methyl-3H-thiophene-2-carboxylic acid (300 mg, 2.08 mmol) and cyclohexylmethanamine (260 mg, 2.29 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

5-bromo-N-(cyclohexylmethyl)thiophene-2-carboxamide (TN19). To synthesize this compound 5-bromothiophene-2-carboxylic acid (300 mg, 1.45 mmol) and cyclohexylmethanamine (180 mg, 1.59 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)-1-methyl-pyrrole-2-carboxamide (TN20). To synthesize this compound 1-methylpyrrole-2-carboxylic acid (300 mg, 2.40 mmol) and cyclohexylmethanamine (300 mg, 2.64 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)furan-2-carboxamide (TN21). To synthesize this compound furan-2-carboxylic acid (300 mg, 2.68 mmol) and cyclohexylmethanamine (330 mg, 2.94 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)-5-methyl-3H-furan-4-carboxamide (TN22). To synthesize this compound 5-methyl-3H-furan-4-carboxylic acid (300 mg, 2.36 mmol) and cyclohexylmethanamine (290 mg, 2.60 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-cyclohexyl-3-(2-methylcyclohexyl)propanamide (TN23). To synthesize this compound 3-(2-methylcyclohexyl)propanoic acid (300 mg, 1.76 mmol) and cyclohexylmethanamine (190 mg, 1.94 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)-3-(2-methylcyclohexyl)propanamide (TN24). To synthesize this compound 3-(2-methylcyclohexyl)propanoic acid (300 mg, 1.76 mmol) and cyclohexylmethanamine (220 mg, 1.94 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)-2-methyl-benzamide (TN25). To synthesize this compound 2-methylbenzoic acid (300 mg, 2.20 mmol) and cyclohexylmethanamine (270 mg, 2.42 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-cyclohexyl-3-(o-tolyl)propanamide (TN26). To synthesize this compound 3-(o-tolyl)propanoic acid (300 mg, 1.83 mmol) and cyclohexylmethanamine (200 mg, 2.01 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

N-(cyclohexylmethyl)-3-(o-tolyl)propanamide (TN27). To synthesize this compound 3-(o-tolyl)propanoic acid (300 mg, 1.83 mmol) and cyclohexylmethanamine (230 mg, 2.01 mmol) were included in the previously described carbodiimide mediated amide formation reaction.

Plant Culture, Treatment Application and Data Collection

Greenhouse trials were established at the University of Tennessee (35.98 N, 83.91W) in 2012 to evaluate the herbicidal activity of Series 1 and Series 2 compounds described above.

Herbicidal activity was evaluated on corn (*Zea mays* L.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), field bindweed (*Convolvulus arvensis* L.), barnyardgrass (*Echinochloa crus-gali* (L.) P.Beauv.), velvetleaf (*Abutilon theophrasti* Medik.), and redroot pigweed (*Amaranthus retroflexus* L.). Corn was included to observe herbicidal activity on a major crop species (Zimdahl 2007). The weed species were selected to include a mix of common small and large seeded monocot and dicot species (DiTomaso 2007). Four corn seeds were planted in the center of 23 cm diameter greenhouse pots (XAM09000, Dillen Products/Myers Industries Inc., Middlefield, OH) containing a potting media (Pro-Mix BX Miycorrhizae, Premier Tech Horticulture Inc., Quakertown, PA). Weed species were surface seeded in clusters by species around the margin of each greenhouse pot. Plants were allowed to grow for 12 days before

application of experimental compounds. On the date of treatment application corn plants averaged 15 cm in height. Weed species height ranged from 3 to 6 cm. After application, plants were watered daily and kept under natural light conditions for 10 days for evaluation. No additional fertility treatments were applied during the course of each study.

All compounds were applied postemergence at a rate of 250 g ha^{-1}. This rate was selected because application of **TN1** at rates less than 250 g ha^{-1} exhibited minimal herbicidal activity in preliminary experiments (data not presented). The 250 g ha^{-1} rate is similar to the maximum recommended use rate of 224 g ha^{-1} for **TDZ** when used for cotton defoliation (Anonymous 2012). Series 1 and Series 2 compounds were evaluated in separate experiments, with each series repeated in time and space. Initial application of Series 1 compounds occurred May 20th, 2012 and May 30th, 2012 for the first and second experimental runs, respectively. Air temperature at application during these experiments ranged from 32 to 35°C, with humidity measuring 52 to 54%. Initial application of Series 2 compounds occurred on June 25th, 2012 and September 8th, 2012 for the first and second experimental runs, respectively. Air temperature at application ranged from 33 to 35°C with humidity averaging 52%.

Compounds were dissolved in 3 mL of acetone before being added to 32 mL of deionized water. Crop oil concentrate (Helena Chemical Company, Collierville, TN) was added at 1 percent volume-to-volume and agitated by hand to form a spray solution. The non-treated check solution was a mixture of 3 mL acetone, 32 mL deionized water, and 1 percent volume to volume of crop oil concentrate. Spray solutions were agitated again before application to the plant species using an enclosed sprayer chamber (Generation III track sprayer. DeVries Manufacturing, Hollandale, MN) at 215 L ha^{-1} through an 8004 EVS nozzle (TeeJet, Wheaton, IL). Herbicidal activity was quantified by visually measuring injury to each plant species 10 days

after treatment on a 0 (i.e., no injury) to 100 % (i.e., complete plant death) scale relative to a non-treated check. Injury was characterized by the presence of chlorosis, necrosis, or epinasty on foliar tissue. Maximum plant height was also recorded for each species and analyzed as percent change from the non-treated check.

Series 1 and Series 2 compounds were evaluated in separate experiments. Each was designed as a randomized complete block with three replications and repeated in time during 2012. Injury and plant height data were arcsine transformed prior to being subjected to analysis of variance in SAS using expected means square values described by McIntosh (1983). Interpretations of non-transformed and transformed data were not different from one another; thus, non-transformed means are presented for clarity. Fisher's protected least significant difference test was used for mean separation in all experiments at $\alpha = 0.05$.

RESULTS

Herbicidal Activity of Series 1 Analogs

No treatment-by-experimental run interactions were observed in injury data; therefore, data from each experimental run were pooled for analysis. Corn injury was significantly higher with **TN1** (7%) than all other treatments (0 to 3%). **TDZ**, **TN1**, and **TN8** application resulted in the greatest injury to large crabgrass (37, 21, and 15%, respectively). However, large crabgrass injury with **TN1** and **TN8** was not significantly different from the other Series 1 compounds tested (0 to 8% injury). Both **TDZ** and **TN1** injured field bindweed 60%. Injury with the other compounds ranged from 0 to 42 %. Redroot pigweed injury was highest with **TDZ** (96%), significantly greater than **TN1** (65%). All other Series 1 compounds injured redroot pigweed similarly (2 to 22%). Responses on velvetleaf were similar to those observed on redroot pigweed

with **TDZ** and **TN1** resulting in the most injury (80% and 56% respectively). No differences in barnyardgrass injury were detected among treatments with injury ranging from 0 to 23% (Table 1.1).

No differences in the height of corn, large crabgrass, field bindweed, barnyardgrass, or redroot pigweed were detected due to treatment with Series 1 compounds. However, application of **TN1** and **TDZ** reduced velvetleaf height 58 to 60% while velvetleaf heights increased 2 to 41% after treatment with other Series 1 compounds (data not presented).

Herbicidal Activity of Series 2 Analogs

Significant treatment-by-experimental run interactions were detected in large crabgrass injury data. Therefore, data from each experimental run were analyzed separately. In the first experimental run **TDZ** caused significantly more injury (62%) to large crabgrass than any other Series 2 compound (<2%). During the second experimental run no differences in large crabgrass injury were observed between treatments. **TDZ** application only resulted in 17% injury and was not significantly different from the non-treated check (Table 1.2).

No treatment-by-experimental run interactions were detected in corn, field bindweed, barnyardgrass, redroot pigweed, or velvetleaf injury data. Therefore, experimental runs were combined for analysis. Field bindweed injury was greatest with **TDZ** (39%) and **TN22** (19%) while no other Series 2 compound tested injured field bindweed greater than 14%. A similar trend was present in velvetleaf injury data as **TDZ**, **TN1**, and **TN22** application caused the greatest injury (80, 35, and 23%, respectively). All other Series 2 compounds tested resulted in <18% velvetleaf injury, statistically similar to the non-treated check. **TDZ** injured redroot

pigweed 93% compared to only 3 to 35% injury for the other Series 2 compounds tested. No differences in barnyardgrass injury were detected among treatments (Table 1.3).

Treatment-by-experimental run interactions were not significant in plant height data collected during evaluations of Series 2 compounds; thus, data were combined. No significant differences in plant height data were detected following application of Series 2 compounds regardless of plant species (data not presented).

DISCUSSION

This experiment supports previous literature that **TN1** is a herbicidally active compound. Arndt (1979) reported that **TN1** exhibited herbicidal activity on *Sinapis*, *Solanum*, *Beta*, *Gossypium*, *Lolium* and *Setaria* species when applied preemergence and postemergence at 2 kg ha^{-1}. Similarly, Arndt (1979) observed herbicidal activity on *Hordeum* and Z*ea* with preemergence applications of **TN1** at the same rate (Arndt, 1979). Our findings indicate that **TDZ** and **TN1** exhibit herbicidal activity on redroot pigweed and velvetleaf at a rate safe for use in corn (250 g ha^{-1}). Future research should evaluate weed control efficacy and crop tolerance with TDZ and TN1 applications on other species.

Series 2 compounds with a similar chemical structure to **TN1**, such as **TN12** and **TN14**, did not elicit responses similar to **TN1** in the current study. The position of the sulfur in the thiadiazole ring is the only difference between **TN12** and **TN1**; while **TN14** contains a nitrogen atom rather than a sulfur atom in the ring structure (Figure 1.5). Sulfur has a lower electronegativity than nitrogen so it is possible that simply including sulfur in the ring structure or at this specific position changed **TN12** and **TN14** interactions with in the binding pocket targeted by **TN1**. Hydrogen or disulfide bonding in this pocket may be important to herbicidal

activity. Additionally, the sulfur atom in the thiadiazole ring contains two lone electron pairs and will not bond with hydrogen atoms similar to carbon or nitrogen. Thus, sulfur atoms are poor electron donators and lack steric influence from a substituent hydrogen. Our findings would suggest that herbicidal activity seems to be dependent on a molecule containing a poor electron donator at the position adjacent to the carboxamide group.

While less herbicidally active than **TDZ** or **TN1**, the only other compound to show moderate herbicidal activity in this research was **TN22** (Figure 1.6). One of the cyclic bases of this compound consists of a furan group rather than the thiadiazole group found in **TN1** (Figure 1.6). Applications of **TN22** resulted in moderate injury to velvetleaf (23%) and field bindweed (19%), statistically greater than non-treated check plants.

It should also be noted that **TDZ**, **TN1** and **TN22** were more active on dicot species such as field bindweed and velvetleaf than the monocot species tested. Fuchs (1968) observed a similar response following applications of the naturally occurring cytokinin, kinetin, to pea (*Pisum sativum* var. Alaska), dwarf pea (*Pisum sativum* var. Progress No. 9) radish (*Raphanus sativus* var. Early Scarlet Globe), bean (*Phaseolus vulgaris* var. Tendergreen Improved), cucumber (*Cucumis sativus* var. Boston Pickling), corn (*Zea mays* var. Golden Bantam), wheat (*Triticum aestivum*), oat (*Avena sativa* var. Victoria), and barley (*Hordcum vulgare* var. Himalaya). Increases in ethylene production following kinetin application were observed with pea, radish cucumber, and corn but not wheat, oat, barley and dwarf pea. Similarly, Suttle (1986) reported that corn plants treated with **TDZ** produced less ethylene than cotton or sunflower (*Helianthus annuus* cv NK 265). Lower injury to monocot species in our experiment may be the result of these synthetic cytokinins failing to sufficiently induce ethylene to injurious concentrations; however, ethylene concentrations after treatment application were not measured

in our research. It is well known that auxin-mimic herbicides injure dicot species more than monocots and also signal ethylene production in susceptible species (Grossmann 2009). The biochemical pathways of cytokinin and auxins production are influenced by one another (Hwang et al. 2012). Future research should explore the use of synthetic cytokinins and auxin-mimic herbicides alone and in combination with one another for weed management.

None of the analogs tested in this experiment induced injury similar to that of **TDZ**. However, **TDZ** was highly active on problematic weeds of corn (velvetleaf and redroot pigweed) and did not result in corn injury. Future research should evaluate crop tolerance and weed control with **TDZ** as it could provide growers a new mode of action for weed management.

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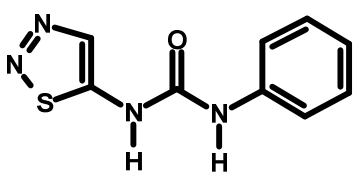
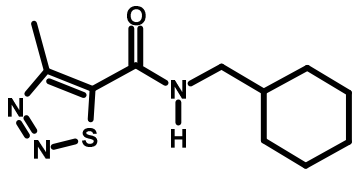
APPENDIX

TABLES AND FIGURES



thidiazuron (TDZ)



N-(cyclohexylmethyl)-4-methyl-thiadiazole-5-carboxamide (**TN1)**

Figure 1.1: Chemical structure of thidiazuron (**TDZ**) and N-(cyclohexylmethyl)-4-methyl-thiadiazole-5-carboxamide (**TN1**) evaluated in greenhouse experiment at the University of Tennessee (Knoxville, TN) in 2012.

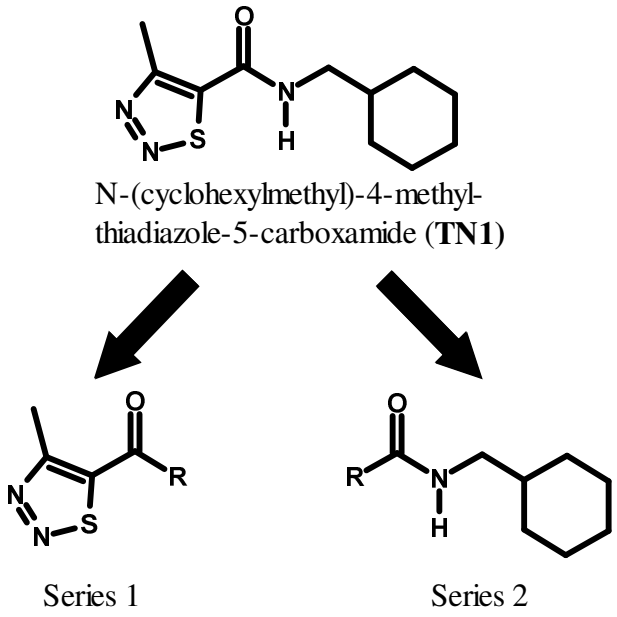


Figure 1.2: Comparison of general chemical structures of N-(cyclohexylmethyl)-4-methyl-thiadiazole-5-carboxamide (**TN1**) and Series 1 and Series 2 compounds at the University of Tennessee (Knoxville, TN) in 2012.

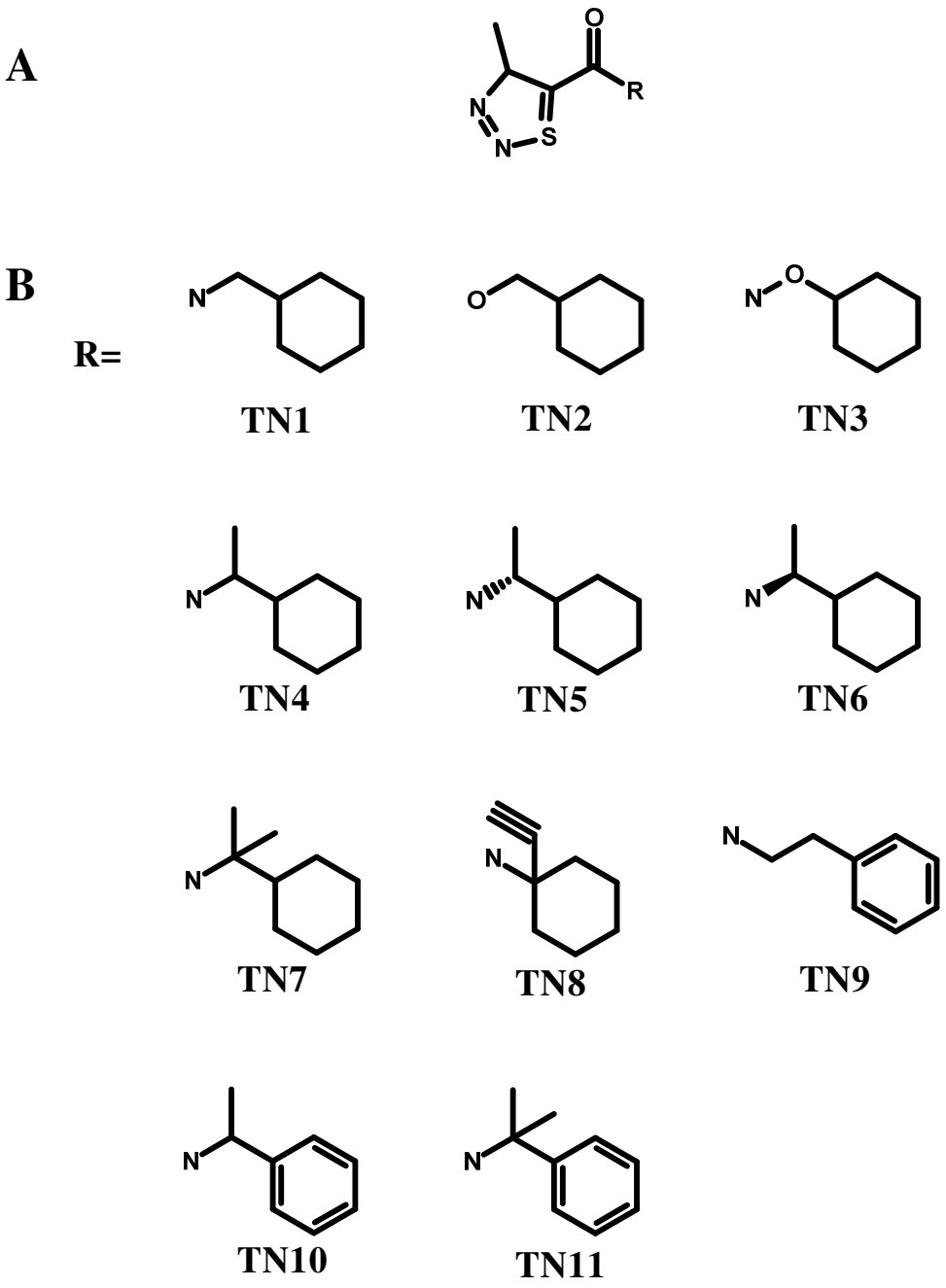
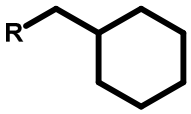


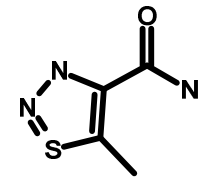
Figure 1.3: Chemical structures of Series 1 compounds evaluated for herbicidal activity on various monocot and dicot plant species in greenhouse experiments at the University of Tennessee (Knoxville, TN) in 2012. Compounds contained (A) a base methylthiadiazole structure and (B) an alcohol or amide structure. Compounds were synthesized using a carbodiimide mediated amide formation.

A

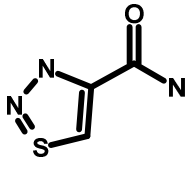


B

R=



TN12



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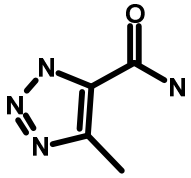
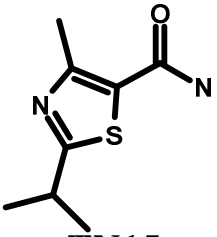


TN14



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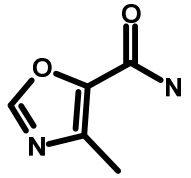
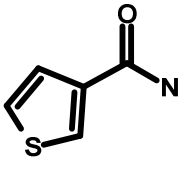


TN16



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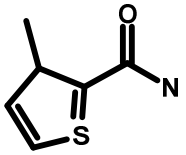

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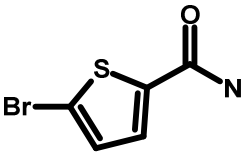
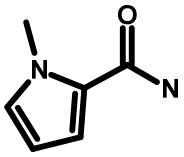
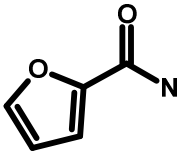


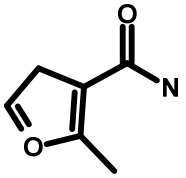
TN19



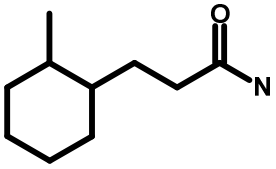
TN20



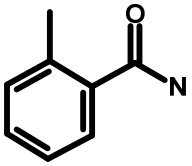
TN21



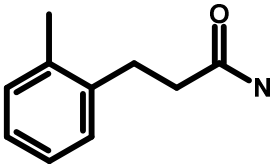
TN22



TN24



TN25



TN27

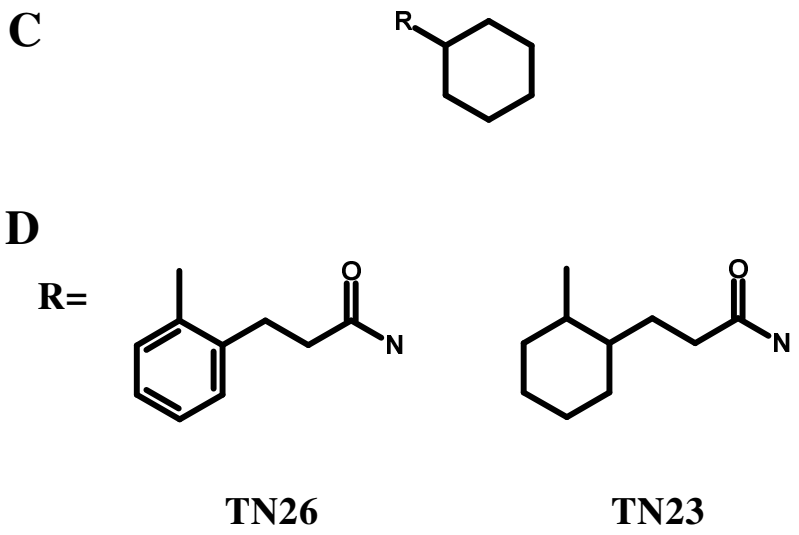


Figure 1.4: Chemical structures of Series 2 compounds evaluated for herbicidal activity on various monocot and dicot plant species in greenhouse experiments at the University of Tennessee (Knoxville, TN) in 2012. Compound contained (A,C) a base cyclohexyl structure and (B,D) an alcohol and amide structure. Compounds were synthesized using a carbodiimide mediated amide formation.

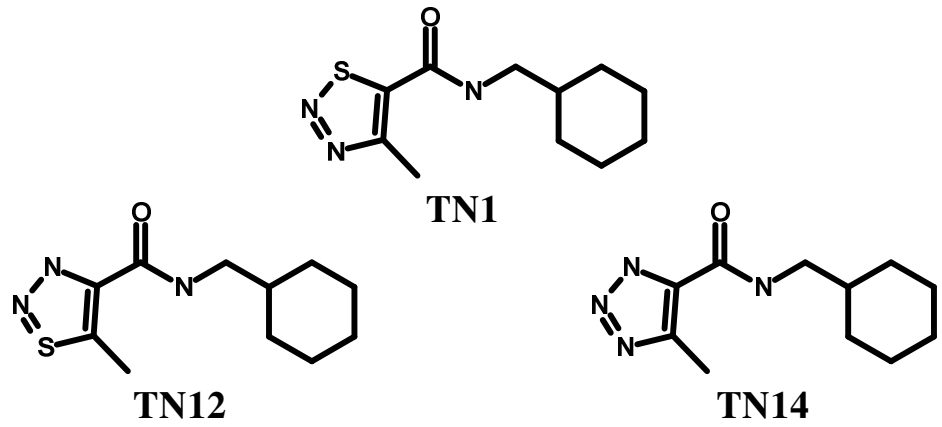
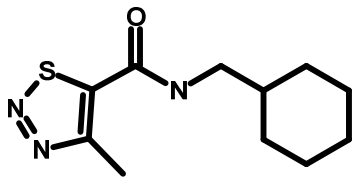
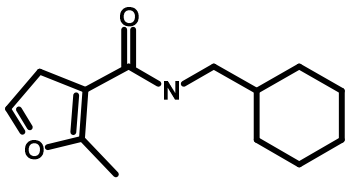


Figure 1.5: Comparison of general chemical structures of N-(cyclohexylmethyl)-4-methyl-thiadiazole-5-carboxamide (**TN1**), N-(cyclohexylmethyl)-5-methyl-1$l^4-thia-2,3-diazacyclopenta-1,4-diene-4-carboxamide (**TN12**), and N-(cyclohexylmethyl)-4-methyl-1H-triazole-5-carboxamide (**TN14**) evaluated for herbicidal activity on various monocot and dicot plant species in greenhouse experiments at the University of Tennessee (Knoxville, TN) in 2012.



TN1



TN22

Figure 1.6: Comparison of general chemical structures of N-(cyclohexylmethyl)-4-methyl-thiadiazole-5-carboxamide (**TN1**) and N-(cyclohexylmethyl)-5-methyl-3H-furan-4-carboxamide (**TN22**) evaluated for herbicidal activity on various monocot and dicot plant species in greenhouse experiments at the University of Tennessee (Knoxville, TN) in 2012.

Table 1.1: Injury to corn (*Zea mays*), large crabgrass (*Digitaria sanguinalis*), field bindweed (*Convolvulus arvensis*), redroot pigweed (*Amaranthus retroflexus*), and velvetleaf (*Abutilon theophrasti*) and 10 days after treatment with Series 1 compounds at 250 g ha^{-1}. Means represent the results of two greenhouse experiments conducted at the University of Tennessee (Knoxville, TN) during 2012.

	Injury[a]				
Compound	Corn	Large crabgrass	Field bindweed	Redroot pigweed	Velvetleaf
	%				
TN1	7	21	60	65	56
TN2	2	3	2	3	3
TN3	0	0	10	2	14
TN4	0	7	22	4	8
TN5	1	0	18	4	8
TN6	3	1	42	20	24
TN7	0	8	10	22	16
TN8	0	15	19	3	1
TN9	1	0	23	17	4
TN10	0	8	14	7	2
TN11	2	3	18	13	3
TDZ	2	37	60	96	80
$LSD_{0.05}$	3	22	35	23	24

[a] Injury was evaluated using a 0 (i.e., no injury) to 100% (i.e., complete plant death) scale relative to a non-treated check

Table 1.2: Injury to large crabgrass (*Digitaria sanguinalis*) 10 days after treatment with Series 2 compounds at 250 g ha^{-1} in greenhouse experiments conducted at the University of Tennessee (Knoxville, TN) during 2012.

	Large Crabgrass Injury[a]	
Compound	Run 1	Run 2
	——————%——————	
TN12	0	0
TN13	0	0
TN14	0	0
TN15	0	3
TN16	2	0
TN17	0	0
TN18	0	0
TN19	0	0
TN20	0	25
TN21	0	0
TN22	0	23
TN23	0	0
TN24	0	0
TN25	0	0
TN26	0	0
TN27	0	0
TDZ	62	17
$LSD_{0.05}$	5	NS

[a] Injury was evaluated using a 0 (i.e., no injury) to 100% (i.e., complete plant death) scale relative to a non-treated check

NS = non-significant

Table 1.3: Corn (*Zea mays*), field bindweed (*Convolvulus arvensis*), barnyardgrass (*Echinochloa crus-gali*), redroot pigweed (*Amaranthus retroflexus*), and velvetleaf (*Abutilon theophrasti*) injury 10 days after treatment with Series 2 compounds at 250 g ha^{-1}. Means represent the results of two greenhouse experiments conducted at the University of Tennessee (Knoxville, TN) during 2012.

	Injury[a]				
Compound	Corn	Field bindweed	Barnyardgrass	Redroot pigweed	Velvetleaf
	%				
TN12	7	3	3	25	35
TN13	0	2	0	8	8
TN14	0	0	2	3	0
TN15	0	2	2	7	5
TN16	11	9	3	13	18
TN17	0	0	1	15	3
TN18	3	0	0	8	8
TN19	0	3	2	8	3
TN20	0	14	10	22	5
TN21	0	2	0	7	12
TN22	3	19	8	8	23
TN23	8	1	0	5	15
TN24	0	0	2	3	7
TN25	0	0	0	23	15
TN26	10	2	3	0	10
TN27	1	0	1	8	12
TDZ	0	39	13	93	80
$LSD_{0.05}$	NS	16	NS	15	18

[a] Injury was evaluated using a 0 (i.e., no injury) to 100% (i.e., complete plant death) scale relative to a non-treated check
NS = non-significant

CHAPTER II

HERBICIDAL ACTIVITY OF HETEROCYCLIC DICHLOBENIL ANALOGS

This chapter is based on a paper to be submitted for publication by Joseph W. Thomas, Gregory R. Armel, Michael D. Best, James T. Brosnan, William E. Klinegman, Dean A. Kopsell, Heidi E. Bostic, Jose J. Vargas, and Chi-Linh Do-Tahnh.

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My contributions to this paper include (i) conducting the experiments, (ii) collecting, processing and collaborating on data analysis and interpretation, (iii) reading literature, (iv) and collaborating on the manuscript.

ABSTRACT

Heterocyclic changes in the chemical structure of existing herbicides may provide new options for weed management. Dichlobenil (2,6-dichlorobenzonitrile) is a preemergence (PRE) herbicide that inhibits cellulose biosynthesis in susceptible plant species. Pyridine (3,5-dichloropyridine-4-carbonitrile) and pyrimidine analogs of dichlobenil (2,4-dichloropyridine-3-carbonitrile; 4,6-dichloropyrimidine-5-carbonitrile) were synthesized or purchased from commercial suppliers and evaluated for weed control in ornamental production. Non-formulated 2,6-dichlorobenzonitrile (the active ingredient in the commercial herbicide dichlobenil) was included for comparison. All compounds were applied PRE at 1, 5, and 10 kg ha^{-1} to large crabgrass (*Digitaria sanguinalis* (L.) Scop.), common purslane (*Portulaca oleracea* L.), and giant foxtail (*Setaria faberi* Herrm.). Phytotoxicity potential of the compounds towards ornamental species was assessed using Japanese holly (*Ilex crenata* Thunb.). All dichlobenil analogs were safe when applied to Japanese holly. The pyrimidine analog (4,6-dichloropyrimidine-5-carbonitrile) controled large crabgrass and common purslane similar to the active ingredient in dichlobenil at all rates evaluated. Additional research should be performed to determine if this pyrimidine analog inhibits cellulose biosynthesis at sites of action similar to dichlobenil.

INTRODUCTION

The prevalence of herbicide resistance in row crop agriculture is leading to increased concerns over similar resistance development in ornamental horticulture production systems. It is well established that widespread use of herbicides with similar modes of action will increase selection pressure for herbicide resistant weed biotypes. Currently, there are 397 biotypes across 217 weed species that exhibit herbicide resistance (Heap 2013). Glyphosate resistance is the largest herbicide resistance concern because of extensive and diverse uses of the herbicide glyphosate (Powles 2008). Biotypes of annual bluegrass (*Poa annua* L.), goosegrass (*Eleusine indica* (L.) Gaertn.), giant ragweed (*Ambrosia trifida* L.), and Palmer amaranth (*Amaranthus palmeri* S.Wats.) resistant to glyphosate have been identified in Tennessee alone (Brosnan et al. 2012, Mueller et al. 2011, Norsworthy et al. 2010, Steckel et al. 2008). Therefore, the search for new herbicide chemistries that target novel sites of action (or known sites of action with few instances of resistance) is critical for developing future sustainable weed management programs in both row crop and ornamental horticulture production systems.

In contrast to glyphosate, only one weed species has developed resistance to inhibitors of cellulose biosynthesis. A biotype of Pollacci barnyardgrass (*Echinochloa erecta* (Pollacci) Pignatti) resistant to quinclorac was identified by Tabacchi et al. (2004). However, there are reports that quinclorac does not inhibit cellulose biosynthesis in barnyardgrass (Tresch and Grossmann 2003). The exact biochemical mechanisms involved in cellulose biosynthesis inhibition have yet to be clearly determined (Delmer and Amor 1995). Other researchers have examined genes associated with cellulose biosynthesis pathways to further understand this mechanism of herbicidal action in whole plants. For example, Sabba and Vaughn (1999) illustrated that herbicides can affect multiple sites within the cellulose biosynthesis pathway

decreasing the possibility of herbicide resistance development. More specifically, other researchers concluded that the complexity of the cellulose synthase gene family associated with the cellulose biosynthesis pathway has made it more difficult for herbicide resistance to develop in comparison to other herbicidal modes of action (Burn et al. 2002).

Dichlobenil is a preemergence (PRE) benzonitrile herbicide first developed in the 1960's that inhibits cellulose biosynthesis in susceptible broadleaf, grass, and sedge species (Anonymous 2012, Koopman and Daams 1960, Koopman and Daams 1962). Dichlobenil is registered for weed control in fruits, nuts, woody ornamentals and some plantation trees species, as well as in certain non-cropland areas (Anonymous 2012, Senseman 2007). Dichlobenil prevents incorporation of glucose into cellulose structures within plant tissues, primarily by inhibiting the movement and proper formation of the cellulose synthase protein complex (CESA6) needed for cellulose synthesis (Senseman 2007, Heim et al. 1998, DeBolt et al. 2007).

Modification of commercially available herbicides through the introduction of heterocyclic motifs (i.e., cyclic ring structures containing atoms other than carbon) has been beneficial in discovering new agents that offer improved weed control and environmental safety. Carbon atoms [as well as adjoining hydrogen(s)] in aromatic ring structures can be replaced with atoms that contain charges and/or lone pairs such as nitrogen, a positively charged oxygen or sulfur atom, or a boron-hydrogen group (Katritzky et al. 2010). New herbicides have been developed by performing these types of heterocyclic changes to commercially available herbicide molecules. For example, dicamba is a benzoic acid auxin-mimic herbicide first patented in 1958 (Richter 1958). Picloram, aminopyralid and clopyralid are pyridine-carboxylic acid auxin-mimic herbicides introduced in the early 1960s (Senseman 2007, Johnston et al. 1964). While both the benzoic acid and pyridine-carboxylic acid families contain two chlorine

atoms and a carboxylic acid substituent group, pyridine-carboxylic acid herbicides (such as picloram, aminopyralid and clopyralid) contain a nitrogen atom within their chemical ring structure that is not present in benzoic acids. This addition of a nitrogen atom to the chemical ring structure resulted in these new herbicides that offer comparable or broader spectrum weed control at lower application rates than dicamba (Senseman 2007, Roeth 1979). Additionally, picloram, aminopyralid and clopyralid have lower mammalian toxicity and are less volatile than dicamba (Senseman 2007).

Introducing heterocyclic groups to dichlobenil may create new structures with improved herbicidal activity that allow for enhanced weed control. However, data describing the herbicidal activity of heterocyclic dichlobenil analogs have not been reported in the scientific literature. Thus, the objective of this research was to design, synthesize, and purchase heterocyclic analogs of dichlobenil and to evaluate these compounds in comparison to a dichlobenil standard for weed control and phytotoxicity when applied over the top of a woody ornamental species, Japanese holly (*Ilex crenata* Thunb.).

MATERIALS AND METHODS

Herbicidal activity of 2,6-dichlorobenzonitrile (dichlobenil), was compared to the following analogs: 3,5-dichloropyridine-4-carbonitrile (**T1**); 2,4-dichloropyridine-3-carbonitrile (**T2**); and 4,6-dichloropyrimidine-5-carbonitrile (**T3**) (Figure 2.1). The **T1** and **T3** compounds were obtained through commercial sources (Sigma Aldrich Co. St. Louis, MO; Activate Scientific Corp. Prien, Germany). The **T2** compound was synthesized using the Sandmeyer reaction (Anderson et al. 1998, Strachan et al. 1998, Yonezawa et al. 2004). NMR spectra of

synthetic compounds were obtained using a Varian Gemini 300 MHz spectrometer (Agilent Technologies, Santa Clara, CA).

Synthesis of 2,4-dichloropyridine-3-carbonitrile (**T2, Scheme 2.1**) was initiated when the starting material, 2,4-dichloropyridin-3-amine (1 g, 6.2 mmol) (Ark Pharm, Inc. Libertyville, IL), was dissolved in 5 mL of acetonitrile in a 250 mL round-bottomed flask. Deionized water (20 mL) was then added to the solution, followed by 3 mL concentrated hydrochloric acid. The solution was cooled to 0°C and sodium nitrite (8.6 g, 12.4 mmol) was added. The solution was then allowed to stir for thirty minutes before being warmed to 20°C and stirred for an additional two hours. At this point, a solution of potassium cyanide (1.21 g, 18.6 mmol) and cupric cyanide (0.61 g, 6.8 mmol) dissolved in 20 mL of deionized water was prepared and added drop wise to the reaction using a dropping funnel. Following addition, the resulting solution was stirred overnight at 20°C, after which it was poured into a separatory funnel and extracted with ethyl acetate (2 x 45 mL). The combined ethyl acetate layers were dried with magnesium sulfate, filtered and the solvent was removed by a rotary evaporator under vacuum (Buchi R-114. Buchi Labortechnik AG. Flawil, Switzerland). The resulting crude product was purified using a flash chromatography column with gradient elution of 3-10% methanol / dichloromethane. This provided **T2** as a light brown solid (52 mg, 0.05% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δH, 5.81, J = 6 Hz; d, 7.23, J = 9 Hz.

Research was conducted in a greenhouse at the University of Tennessee (35.98 N, 83.91W) during 2011 and 2012 to evaluate the herbicidal activity of each experimental compound (**T1, T2, T3**) compared to non-formulated dichlobenil. Compounds were applied to Japanese holly (*Ilex crenata*), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), giant foxtail (*Setaria faberi* Herrm.), and common purslane (*Portulaca oleracea* L.). These species were

selected to evaluate ornamental tolerance and weed susceptibility to **T1, T2, T3,** and dichlobenil. Each plant species was established in separate 10.2 cm x 10.2 cm plastic pots (Dillen Products/Myers Industries, Inc. Middlefield, OH.) filled with Sequatchie loam soil (fine-loamy, siliceous, semiactive, thermic, and humic Hapludult) with a pH of 5.8 and organic matter content of 2.1 %. This growing medium was blended with a calcined clay based soil conditioner (Turface. Profile Products, LLC. Buffalo Grove, IL) in a 3:1 soil:clay ratio. Japanese holly cuttings were transplanted into pots two months prior to treatment. Japanese holly plants were 14 cm at the time of application. Weed species were surface seeded just prior to treatment and incorporated into the top 2 cm of soil. During the study plants were kept under natural light conditions and watered daily.

All compounds were applied PRE at rates of 1, 5, and 10 kilograms per hectare (kg ha^{-1}). Test substances were dissolved in a mixture of 3 mL acetone and 32 mL deionized water and agitated using a sonicator (CL-18, Fisher Scientific International Inc. Hampton, NH) before application. The non-treated check solution was a mixture of 3 mL acetone and 32 mL deionized water. All suspensions were further agitated by hand immediately prior to treatment in an enclosed sprayer chamber (Generation III track sprayer. DeVries Manufacturing, Hollandale, MN) at 215 liters/hectare through an 8004 EVS nozzle (TeeJet, Wheaton, IL).

Foliar Japanese holly injury was assessed 14, 21, and 28 days after treatment (DAT) on a 0 (i.e., no injury) to 100 % (i.e., complete plant death) scale relative to a non-treated check. Japanese holly injury was characterized by the presence of chlorosis, necrosis, or epinasty on foliar tissue. Weed control was also assessed 14, 21, and 28 DAT using a similar 0 to 100% scale. Treatments were arranged in a 4 x 3 factorial, randomized complete block design with three replications. Factors included four compounds (dichlobenil, **T1**, **T2**, and **T3**) and three

application rates (10, 5, and 1 kg ha^{-1}). The experiment was conducted from 2 November 2011 to 30 November 2011 when air temperature was 35°C and humidity was 48% at application. The experiment was repeated from 20 August 2012 to 17 September 2012 when air temperature was 26% and humidity was 63% at application. Japanese holly injury and weed control data were arcsine transformed prior to being subjected to analysis of variance using the models of McIntosh (1983). Interpretations were not different from non-transformed data; therefore, non-transformed means are presented for clarity. Fisher's protected least significant difference test (α = 0.05) was used for mean separation.

RESULTS

No significant interactions between treatment-by-experimental run were detected in Japanese holly injury or weed control data; thus, data from each experimental run were combined. Japanese holly injury did not vary due to applied treatments; overall injury ranged from only 0 to 7% by 28 DAT (data not presented). Similarly, giant foxtail control only ranged from 0 to 4% by 28 DAT with no differences detected between treatments (Table 2.1).

Large crabgrass control varied due to treatment (Table 2.1). When applied PRE at 10 kg ha^{-1} the **T2** and **T3** analogs controlled large crabgrass 20 and 46% respectively by 28 DAT (Table 2.1). Large crabgrass control with **T2** and **T3** was similar to the herbicide dichlobenil (46%) on this assessment date. No significant differences in large crabgrass injury were detected between the 1 and 5 kg ha^{-1} application rates of dichlobenil although both rates injured large crabgrass less than 10 kg ha^{-1}. A similar response was observed with the 1 and 5 kg ha^{-1} rates of the pyrimidine analog (**T3**). No differences were detected among the three application rates of

the two pyridine analogs (**T1** and **T2**) as large crabgrass injury only ranged from 5 to 20% 28 DAT.

The pyrimidine analog (**T3**) controlled common purslane similar to dichlobenil at all rates. By 28 DAT, common purslane control ranged from 62 to 70% with **T3** or dichlobenil applied PRE at 10 kg ha^{-1} herbicide. Reducing the rates of **T3** and dichlobenil to between 1 to 5 kg ha^{-1} resulted in 20% or less control of common purslane. Neither pyridine analog (**T1, T2**) controlled common purslane greater than 15% regardless of rate (Table 2.1).

DISCUSSION

Japanese holly tolerance to dichlobenil in this study supports current labeling for use on holly species (Anonymous 2012). However, weed control (e.g., large crabgrass, common purslane) with dichlobenil in this study was lower (< 20%) than would be expected with an application at the labeled rate of 5 kg ha^{-1}. Poor weed control with dichlobenil at 5 kg ha^{-1} could be attributed to the fact that the active ingredient was not applied as a commercial formulation in this study; rather, technical grade dichlobenil was agitated in mixture with deionized water and acetone before being applied to plants. Furthermore, even formulated dichlobenil can be volatile (Parochetti et al. 1971), which could also explain the reduced activity observed with technical grade dichlobenil rates as high as 10 kg ha^{-1}. Future research should compare ornamental tolerance and weed control following treatment with the heterocyclic analogs in the current study (**T1**, **T2**, **T3**) to dichlobenil under conditions that reduce the risk of volatilization, such as applications to dry soil (Parochetti et al. 1971) or incorporating compounds into soil after application (Barnsley and Rosher 1961).

The pyridine analogs (**T1**, **T2**) were also safe to Japanese holly but did not control large crabgrass, common purslane, or giant foxtail greater than 20% when applied PRE. It is not clear from these data why pyridine analogs of dichlobenil did not control these weed species similar to dichlobenil or the pyrimidine analog (**TN3**). This response is similar to those reported by Beeler et al. (2012) who observed greater control of trumpetcreeper (*Campsis radicans*) with the pyrimidine-carboxylic acid herbicide, aminocyclopyrachlor-methyl, compared to the pyridine-carboxylic acid herbicide, aminopyralid. In additional experiments, aminocyclopyrachlor-methyl controlled largeleaf lantana (*Lantana camara*) more than both aminopyralid and another pyrimidine-carboxylic acid, fluroxypyr (Ferrell et al. 2012). In pharmaceutical research, inhibition of lipid peroxidation was greater with pyrimidine analogues of acetaminophen than with pyridines (Nam et al. 2009).

The pyrimidine analog (**T3**) was safe for use on Japanese holly and controlled large crabgrass and common purslane similar to dichlobenil at all rates evaluated with preemergence application. Additional research should be conducted to determine if **T3** inhibits cellulose biosynthesis and whether the specific sites of action targeted by **T3** are similar to the commercial standard dichlobenil using the methods described by DeBolt et al. (2007). We hypothesize that the mode of action of **T3** will be similar to dichlobenil considering that heterocyclic analogs of commercial herbicides typically exhibit the same mode of action. For example, the benzoic acid and pyridine-carboxylic acid herbicide families are both considered synthetic auxin herbicides despite their differences in heterocyclic structure (Senseman 2007). Similarly, ring structures of the herbicides metsulfuron-methyl and nicosulfuron differ from one another but both are classified as acetolactate synthase inhibitors (Senseman 2007). Dichlobenil and **T3** may be both classified as inhibitors of cellulose biosynthesis but act at different sites of action in the cellulose

biosynthesis pathway, similar to dichlobenil and isoxaben. Although both are classified as cellulose biosynthesis inhibitors, dichlobenil inhibits the conversion of UDP-glucose to cellulose while isoxaben compromises the conversion of sucrose to UDP-glucose (Sabba and Vaughn 1999). Experiments should also be conducted using a broader range of ornamental and weed species comparing ornamental plant tolerance and weed control with **T3** and dichlobenil. Differences in ornamental tolerance and weed susceptibility between these two compounds could provide new options for weed management, especially if **T3** targets novel sites of action within the cellulose biosynthesis pathway. Furthermore, other analogs of dichlobenil should be examined for herbicidal activity, particularly a triazine analog containing three nitrogen atoms in the ring structure as well as molecules with ring structures containing other atoms, such as oxygen.

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APPENDIX

TABLES AND FIGURES

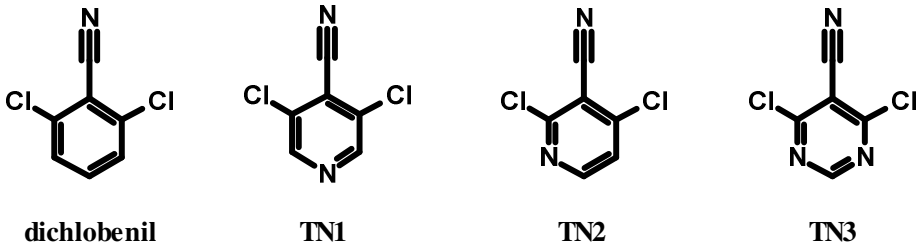

Figure 2.1: Chemical structures of dichlobenil and heterocyclic analogs evaluated as novel herbicides in greenhouse research trials at the University of Tennessee (Knoxville, TN). Compounds pictured include: 2,6-dichlorobenzonitrile (dichlobenil); 3,5-dichloropyridine-4-carbonitrile (**T1**); 2,4-dichloropyridine-3-carbonitrile (**T2**); and 4,6-dichloropyrimidine-5-carbonitrile (**T3**).

Scheme 2.1: Preparation scheme for 2,4-dichloropyridine-3-carbonitrile (**T2**) evaluated for weed control and ornamental tolerance in two combined greenhouse trials at the University of Tennessee (Knoxville, TN) in 2011 and 2012.

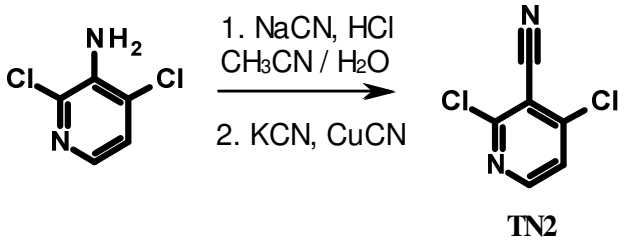

Table 2.1: Large crabgrass (*Digitaria sanguinalis*), common purslane (*Portulaca oleracea)*, and giant foxtail (*Setaria faberi*) control 28 days after treatment with dichlobenil (2,6-dichlorobenzonitrile), and the heterocyclic analogs 3,5-dichloropyridine-4-carbonitrile (**T1**), 2,4-dichloropyridine-3-carbonitrile (**T2**), and 4,6-dichloropyrimidine-5-carbonitrile (**T3**). Means captured responses from two combined glasshouse experiments conducted in 2011 and 2012.

		Weed control[a]		
Compound	Rate	Large crabgrass	Common purslane	Giant foxtail
	kg ha^{-1}	%		
dichlobenil	1	12	3	0
	5	17	20	0
	10	46	70	4
T1	1	5	1	1
	5	8	6	1
	10	13	3	3
T2	1	10	15	0
	5	7	7	0
	10	20	7	0
T3	1	3	5	3
	5	23	18	0
	10	46	62	0
$LSD_{0.05}$	-	27	24	NS

[a] Weed control was evaluated using a 0 (i.e., no control) to 100% (i.e., complete plant death) scale relative to a non-treated check

CONCLUSION

An objective of this research was to synthesize a novel herbicide that could control weeds using a potentially new mode of action. This would help to not only mitigate the rate at which herbicide resistance is increasing but also potentially provide new options for controlling weeds resistant to various herbicide chemistries.

Thidiazuron (**TDZ**) is a synthetic cytokinin that injured problematic weeds of corn when applied POST in our research. Twenty seven compounds similar in structure to **TDZ** were synthesized with effects evaluated following applications to corn (*Zea mays*), large crabgrass (*Digitaria sanguinalis*), field bindweed (*Convolvulus arvensis*), barnyardgrass (*Echinochloa crus-gali*), velvetleaf (*Abutilon theophrasti*), and redroot pigweed (*Amaranthus retroflexus*). Application of **TDZ** and N-(cyclohexylmethyl)-4-methyl-thiadiazole-5-carboxamide (**TN1**) resulted in the greatest injury on each species. Results indicate that exploration of compounds with structural similarity to **TDZ** remains the most likely route towards the discovery of an effective herbicidally active synthetic cytokinin.

The herbicidal activity of heterocyclic analogs of dichlobenil was also evaluated in greenhouse research trials. Pyridine (3,5-dichloropyridine-4-carbonitrile; **TN1**, 2,4-dichloropyridine-3-carbonitrile; **TN2**) and pyrimidine (4,6-dichloropyrimidine-5-carbonitrile; **TN3**) analogs of dichlobenil, were synthesized or purchased from commercial suppliers and evaluated for weed control in ornamental production. Non-formulated 2,6-dichlorobenzonitrile (the active ingredient in the commercial herbicide dichlobenil) was included for comparison. None of the compounds tested significantly injured Japanese holly. Application of the pyrimidine analog (**T3**) controlled large crabgrass and common purslane was similar to dichlobenil at all rates evaluated. However, overall injury with both materials was low. Additional research is required to evaluate the efficacy of this compound for weed control under

an array of environmental conditions as well as to determine if applications target the same mode and site of action as dichlobenil.

While herbicide resistant weed species are a significant challenge to effective weed management, prevalence of herbicide resistance has renewed interest in herbicide discovery. The entire field of weed management will benefit from the continued focus on the discovery of new herbicidal modes of action. Knowledge about herbicidally active compounds identified in these studies may be used to further development of new herbicides and understanding of weed species as a whole.

VITA

Joseph W. Thomas was born in Jackson, Mississippi on August 25th, 1988. Joseph was raised in Wilmington, Delaware where he graduated from Concord High School in 2006. He then obtained a Bachelor's of Science studying Wildlife Conservation and Plant Science from the University of Delaware in 2010. He began his Master's of Science degree in 2011 at the University of Tennessee and plans to pursue a Ph.D. after graduation